# Draft data section of the Scientific opinion on Risk assessment of African swine fever and the ability of products or materials to present a risk to transmit ASF virus


## EFSA Panel on Animal Health and Welfare


## Background

EFSA has been supporting EU Member States and the European Commission in the fight against African Swine Fever (ASF) by providing different scientific opinions covering different aspects of the disease, including the risk of introduction of ASF into the European Union, the role of vectors (ticks), the spread of ASF, standardised data collection on ASF, and updates on the epidemiological situation of ASF in the EU.

Recently, EFSA has been requested to review its previous evaluation (EFSA, 2014) of the ability of different matrices, such as meat and meat products from ASFV-infected pigs and ASFV-contaminated materials, including vegetables, arable crops, hay, straw as well as sawdust, wood chips and similar materials, to transmit ASFV to domestic pigs, and to rank the different matrices on the basis of their level of risk of transmitting the virus.

## Approach taken for this data section

This data section focusses on matrices or products that that are traded legally and that could potentially lead to the transmission of ASFV to domestic pigs, provided that these products would contain ASFV, and that efficient exposure to domestic pigs takes place. Therefore, transport vehicles and tote bags have equally been included.

As a first step, peer-reviewed literature has been searched for experimental infection or virus survival studies that examined the ability of ASFV to survive and remain viable in different matrices, as evidenced by virus isolation. Studies demonstrating only the presence of ASFV-DNA through PCR and not using virus isolation were excluded from the review, as PCR-positive samples do not necessarily contain infectious virus. However, it cannot be excluded that products which are found negative by virus isolation may still contain a small amount of infectious virus. Relying only on studies using virus isolation as detection method could therefore underestimate the survival time of ASFV. However, in most of the identified studies except one (Petrini et al., 2019) that carried out challenge studies on virus isolation-negative samples, inoculation of pigs with these samples did not result in infection. The review includes studies on any of the different ASFV strains, as to date there is no scientific evidence that suggests that certain strains would survive better than others. For details of the search carried out see the protocol in the Annex.

For categories of matrices that the Animal Health and Welfare (AHAW) panel considers to be at risk of contamination with ASFV, but for which no data have been identified in the literature review, information on the matrix production or processing parameters was collated from legal documents and peer-reviewed literature, to understand if the production process or the processing of the matrices would allow the virus to remain viable, should the matrices have been contaminated with ASFV before its production and/or processing.

Studies regarding virus isolation from live animals were not included in the assessment. This has been the subject of other reviews (EFSA, 2014; Stahl et al., 2019). Further, the moving of live animals is strictly regulated and is not within the scope of this risk assessment

The data section has been structured into two parts: the first is related to survival of ASFV in products derived from ASFV-infected domestic pigs; the second summarises data regarding ASFV-survival in other matrices that may become contaminated with ASFV through direct contact with ASFV-infected animals and/or through indirect contact (e.g. excretions) with infected ASFV-animals.

# 1. ASFV survival in products derived from infected pigs

## 1.1. Unprocessed meat

### 1.1.1. Pig carcases

No data on ASFV survival in whole pig carcases, i.e. the body of a pig after slaughter and dressing, have been identified in the literature review. Data from studies on survival of ASFV in parts of a carcase, such as bones, fresh meat (cuts) and viscera of the abdominal and thoracic cavity organs are described in the specific sections below.

### 1.1.2. Fresh pig blood

ASFV has been demonstrated to survive for more than 1 year (525 days) in chilled pig blood (Plowright et al., 1967).

### 1.1.3. Fresh pork meat

ASFV was isolated from fresh whole and ground pork meat stored at 4°C for 2 days (McKercher et al., 1978). No further tests were carried out on these matrices at later stages of the experiment. The amount of blood present in fresh pork meat is only very small once the meat cuts are prepared.

### 1.1.4. Abdominal viscera (heart, intestines, kidney, liver, spleen)

It has been shown, that ASFV can survive in frozen (-18°C) pig heart and kidney for at least 60 days (Sindryakova et al., 2016). No data on ASFV survival in pig intestines have been identified in the literature review. In liver stored at room temperature (23.5°C), ASFV survives for 16 days, while the virus remains viable in frozen liver (-18°C) for at least 60 days (Sindryakova et al., 2016). In frozen spleen (-20°C and -70°C) the virus survives for at least 735 days (Plowright et al., 1967).

### 1.1.5. Pig bones

Mebus et al. (1997) reported that bone marrow collected from experimentally infected pigs tested positive for ASFV immediately after slaughter, evisceration and halving of carcases. No further tests were carried out on this matrix at later stages of this experiment. McKercher et al. (1987) were able to isolate ASFV from bone marrow of Parma hams on day 94, but not on day 123 after the start of the experiment (72 h after slaughter).

### 1.1.6. Pig fat

It has been shown that ASFV can survive in chilled pig fat for at least 735 days (Plowright et al., 1967) and for at least 60 days in frozen (-18°C) pig fat (Sindryakova et al., 2016).

Table 1 Survival time of ASFV in unprocessed meat as reported in literature

| Matrix | Storage temperature (°C) | Humidity range (%) | Maximum number of days infectious virus detected | First ASFV negative observation | Duration of the experiment in days | ASFV Half-life in days | LCI 95%[1] | UCI 95%[2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blood** | Chilled (4°C) | | 525 | | 735 | | | | | Plowright et al., 1967 |
| **Pork meat, whole** | Chilled (4°C) | | 2 | | 2 | | | | pH 5.6 | McKercher et al., 1978 |
| **Pork meat, ground** | Chilled (4°C) | | 2 | | 2 | | | | pH 5.6 | McKercher et al., 1978 |
| **Heart** | Frozen (-18°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| **Spleen** | Frozen (- 20°C) | | ≥735 | | 735 | | | | | Plowright et al., 1967 |
| **Spleen** | Frozen (- 70°C) | | ≥735 | | 735 | | | | | Plowright et al., 1967 |
| **Kidney** | Frozen (-18°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| **Liver** | Room (23.5°C) | | 16 | | 60 | | | | | Sindryakova et al., 2016 |
| **Liver** | Frozen (-18°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| **Pig bones** | nr | | After slaughter[3] | | na | | | | | Mebus et al., 1997 |
| **Pig bones** | | | 94 | 123 | 432 | | | | | McKercher et al., 1987 |
| **Pig fats** | Chilled (5°C) | | ≥720 | | 720 | | | | | Sindryakova et al., 2016 |
| **Pig fats** | Frozen (-18°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval; [3]bone marrow samples were collected immediately after slaughter, evisceration and halving of carcases; nr=not reported; na=not applicable

## 1.2. Processed meat products

The following section contains data from studies of processed meat products, i.e. products that have been subjected to processes that substantially alter the initial 'raw' product, such as heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes. Only categories of meat products that were studied in the experimental infection studies or virus survival studies identified in the literature review are described in section 1.2.

### 1.2.1. Heat-treated processed meat

No viable ASFV was detected once heating had been completed for ham brined and heated to 69°C (McKercher et al., 1978). Canned stew pork produced by using long-term exposure to high temperatures did not yield any viable ASFV during 60 days of storage at 4-6°C or −16 to −20°C (Sindryakova et al., 2016). No data on other types of heat-treated processed meat was identified.

Table 2 Survival time of ASFV in heat-treated processed meat as reported in literature

| Processed product | Temperature range (°C) | Humidity range (%) | Maximum number of days ASFV was detected (last positive observation) | First ASFV negative observation | Duration of the experiment in days | Half-life in days | LCI 95%[1] | UCI 95%[2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Ham brined and heated | 69°C[3] | | na | na | na | | | | No virus was detected once heating had been completed | McKercher et al., 1978 |
| Canned stew pork | Frozen (−16-−20°C) | | 0 | | 60 | | | | The virus was inactivated during the production of canned stew pork[4] | Sindryakova et al., 2016 |
| Canned stew pork | Chilled (4-6°C) | | 0 | | 60 | | | | The virus was inactivated during the production of canned stew pork[4] | Sindryakova et al., 2016 |
| Canned stew pork | Room temperature (20-25°C) | | 0 | | 60 | | | | The virus was inactivated during the production of canned stew pork[4] | Sindryakova et al., 2016 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval; [3]temperature was slowly increased so that in about 3.5 h, the internal temperature of the ham was 69°C; [4]Canned meat was prepared in compliance with the specification of RF State Standards (GOST 32125-2013 Canned stew meat) using long-term exposure to high temperatures; na=not applicable.

### 1.2.2. Non-heat-treated processed meat

All studies about non-heat-treated processed meat identified in the literature review focussed on different cured products.

#### *1.2.2.1. Immersion cured products*

ASFV was detected for at least 60 days (i.e. throughout the duration of the experiment) in frozen (−16 to−20°C) and chilled (4–6°C) corned pork prepared using a wet salting method. In corned pork stored at room temperature, ASFV was last detected on day 16 of the experiment (Sindryakova et al., 2016).

Ham brined and stored at 4°C was found positive for ASFV 2 days when processing was completed (McKercher et al., 1978). No further tests were carried out.

#### *1.2.2.2. Dry-cured products*

Pork belly that had been cured for 14-21 days and pork loin cured for 60 days were found to be positive for ASF virus at least 60 and 83 days, respectively. The first ASFV-negative samples were detected 137 days after initiation of processing the meat (Petrini et al, 2019).

Salami cured for 27 days at temperatures ranging between 22 to 12°C and a humidity between 77-99 % was last tested positive 18 days after the start of the processing; the first negative test was 26 days after the start of processing (Petrini et al, 2019). Salami smoked for 12 h at 32°C and for an additional 12 h at 49°C and a humidity of 72 % tested negative after 30 days after the start of processing (McKercher et al., 1978).

Peperoni sausage smoked for 8h at a temperature of 32.2 to 34.4°C and a humidity of 72 % tested negative after 30 days after the start of processing (McKercher et al., 1978).

Iberian loin cured for 90-130 days was tested negative for ASFV after 112 days of processing. Iberian Ham cured for 365-730 days, Iberian shoulder cured for 240-420 days and Serrano ham cured for 180-365 days tested negative for ASFV 140 days after the start of processing (Mebus et al., 1997).

#### *1.2.2.3. Other cured products*

Frozen dry-salted pork fatback (−16 to−20°C) was ASFV positive for at least 60 days. Chilled dry-salted pork fatback (4-6°C) was ASFV negative at the beginning of the processing (Sindryakova et al., 2016).

Table 3 Survival time of ASFV in non-heat-treated processed meat as reported in literature

| Product category | Processed product | Temperature range (°C) | Humidity range (%) | Maximum number of days ASFV was detected (last positive observation) | First ASFV negative observation | Duration of the experiment in days | Half-life in days | LCI 95% [1] | UCI 95% [2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Immersion cured products | Corned pork | Frozen (−16-−20°C) | | 60 | | 60 | | | | Corned pork was prepared using meat of infected piglets, using a wet salting method | Sindryakova et al., 2016 |
| Immersion cured products | Corned pork | Chilled (4–6°C) | | 60 | | 60 | | | | Corned pork was prepared using meat of infected piglets, using a wet salting method | Sindryakova et al., 2016 |
| Immersion cured products | Corned pork | Room temperature (20–25°C) | | 16 | | 60 | | | | Corned pork was prepared using meat of infected piglets, using a wet salting method. | Sindryakova et al., 2016 |
| Immersion cured products | Ham brined | 4°C | | 2 | | Full processing time= 60 days | | | | No virus was detected beyond processing period | McKercher et al., 1978 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry-cured products | Pork belly | | | 60 | 137 | 137 | | | | Curing time: 14-21 d. ASFV was detected in the pork belly in the final product | Petrini et al, 2019 |
| Dry-cured products | Pork loin | | | 83 | 137 | 137 | | | | Curing time: 60 d. ASFV was detected in the pork loin in the final product | Petrini et al, 2019 |
| Dry-cured products | Salami | Combination of temperatures during curing | | 18 | 26 | 137 | | | | Curing time: 27 d. The virus was not recovered beyond the processing period. | Petrini et al, 2019 |
| Dry-cured products | Salami sausage | Smoked for 12 h at 32°C and for an additional 12 h at 49°C | 72 | 9 | 30 | 30 | | | | Tested negative after 30 days after smoking | McKercher et al., 1978 |
| Dry-cured products | Pepperoni sausage | Smoked for 8h at a temperature of 32.2 to 34.4°C | 72 | 8 | 30 | 30 | | | | Tested negative after 30 days after smoking | McKercher et al., 1978 |
| Dry-cured products | Iberian loin | | | 112 | | Curing time: 90-130 | | | | | Mebus et al., 1997 |
| Dry-cured products | Iberian Ham | Combination of temperatures during curing | | 140 | | Curing time: 365-730 | | | | No viable virus was detected once curing had been completed | Mebus et al., 1997 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry-cured products | Iberian shoulder | | | 140 | | Curing time: 240-420 | | | | | Mebus et al., 1997 |
| Dry-cured products | Serrano ham | | | 140 | | Curing time: 180-365 | | | | | Mebus et al., 1997 |
| Other cured products | Salted pork fatback | Frozen (−16--20°C) | | 60 | | 60 | | | | Viable virus was detected for >60 days (i.e. beyond the duration of the experiment) | Sindryakova et al., 2016 |
| Other cured products | Salted pork fatback | Chilled (4-6°C) | | 0 | | 60 | | | | | Sindryakova et al., 2016 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval; [3]The fatback was processed by dry salting in compliance with the specifications of technology GOST 38-8549 -Products of pork fatback.

### 1.2.3. Casings

Pork sausage casings stored at a mean temperature of 15°C or 12.3°C were found positive for ASFV until the end of the experiment (30 days) (Dee et al., 2018, Stoian et al., 2019). The half-life of the virus in pork sausage casings was estimated to be 13.1 days (CI 95% 11.6-14.6) (Stoian et al., 2019). Casings in medium and stored at 4°C were positive 7 days after the start of the experiment and tested negative for the first time on day 14 (Jelsma et al., 2019).

Table 4 Survival of ASFV in casings as reported in literature

| **Matrix** | **Temperature range (°C)** | **Humidity range (%)** | **Maximum number of days infectious virus was detected** | **First ASFV negative observation** | **Duration of the experiment in days** | **Half-life in days** | **LCI 95%[1]** | **UCI 95%[2]** | **Comment** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|
| Pork sausage casings | Room (12.3 °C (mean)) | 74.1 (mean) | 30 | | 30 | 13.1 | 11.6 | 14.6 | | Stoian et al., 2019 |
| Casings in medium | Chilled (4 °C) | | 7 | 14 | 60 | | | | | Jelsma et al., 2019 |

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|---|---|---|---|---|---|---|---|---|---|---|
| Pork sausage casings | 15°C (mean) | 75 (mean) | 30 | | 30 | | 4.4 | | | Dee et al. 2018 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval

### 1.2.4. Animal by-products for use in feed

Category 3 animal by-products (ABP) destined for use in feed must have undergone one of the processes listed in Chapter III of Annex IV of Regulation 142/2011. These include methods 1 to 5 and 7 as listed in Table 5. For method 7, no standard conditions are prescribed, however, the method should be authorised by the competent authority in the MS.

Table 5 Standard processing methods for Category 3 animal by-products

| **Method** | **Maximum particle size of raw material to be treated** | **Core temperature achieved** | **Minimum time at core temperature** | **Pressure** | **Special details** |
|---|---|---|---|---|---|
| **Method 1 (pressure sterilisation)** | 50 mm | >133°C | 20 minutes without interruption | 3 bars | The pressure must be produced by the evacuation of all air in the sterilisation chamber and the replacement of the air by steam ('saturated steam'); the heat treatment may be applied as the sole process or as a pre- or post-process sterilisation phase; the processing may be carried out in batch or continuous systems |
| **Method 2** | 150mm | >120°C | 50 minutes | | Processing must be carried out in batches |
| **Method 2** | 150mm | >110°C | 120 minutes | | Processing must be carried out in batches |
| **Method 2** | 150mm | >100°C | 125 minutes | | Processing must be carried out in batches |
| **Method 3** | 30mm | >120°C | 13 minutes | | Processing may be carried out in batch or continuous systems |
| **Method 3** | 30mm | >110°C | 55 minutes | | Processing may be carried out in batch or continuous systems |

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| **Method 3** | 30mm | >100°C | 95 minutes | | Processing may be carried out in batch or continuous systems |
| **Method 4** | 30mm | >130°C | 3 minutes | | After reduction the animal by-products must be placed in a vessel with added fat<br>Processing may be carried out in batch or continuous systems |
| **Method 4** | 30mm | >120°C | 8 minutes | | After reduction the animal by-products must be placed in a vessel with added fat<br>Processing may be carried out in batch or continuous systems |
| **Method 4** | 30mm | >110°C | 13 minutes | | After reduction the animal by-products must be placed in a vessel with added fat<br>Processing may be carried out in batch or continuous systems |
| **Method 4** | 30mm | >100°C | 16 minutes | | After reduction the animal by-products must be placed in a vessel with added fat<br>Processing may be carried out in batch or continuous systems |
| **Method 5** | 20mm | >100°C | 60 minutes | | After reduction and before application of the heat treatment, the animal by-products must be heated until they coagulate and then pressed so that fat and water are removed from the proteinaceous material<br>Processing may be carried out in batch or continuous systems |
| **Method 5** | 20mm | >80°C | 120 minutes | | After reduction and before application of the heat treatment, the animal by-products must be heated until they coagulate and then pressed so that fat and water are removed from the proteinaceous material<br>Processing may be carried out in batch or continuous systems |

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|---|---|---|---|---|---|
| **Method 7*** | not defined | not defined | not defined | | Any processing method that has been authorised by the competent authority and has been demonstrated to reduce relevant hazards in the starting material to a level which does not pose any significant risks to public and animal health with the final product complying with specific microbiological standards |

*A list of approved methods in 2018 is available at https://efsa.onlinelibrary.wiley.com/cms/attachment/3483307c-9a2f-436f-8715-082174dd3dfe/efs25314-fig-0003-m.jpg

#### *1.2.4.1 Hydrolysed proteins for use in feed*

No data on ASFV survival in hydrolysed proteins have been identified in the literature review. Hydrolysed proteins must be produced by a process which involves appropriate measures to minimise contamination (Regulation 142/2011, Annex X, chapter II, section 5). According to Hou et al. (2017), the general procedures for the production of hydrolysed proteins from animal products (including by-products) through chemical, enzymatic, or microbial hydrolysis include a heat treatment (pasteurization) (table 6). These general procedures may be modified for peptide production, depending on protein sources and product specifications.

Table 6 Production process of hydrolysed proteins (Hou et al., 2017)

| Hydrolysis | Separation | Decontamination | Further processing |
|---|---|---|---|
| hydrolysis of proteins by cell-free proteases, microorganisms, acids, or bases | centrifugation, filtration, microfiltration | heat-treatment (pasteurization) | drying |

#### *1.2.4.2 Rendered fats for use in feed*

No data on ASFV survival in rendered fats have been identified in the literature review. According to Commission Regulation (EU) No 142/2011, Annex IV Chapter III, rendered fats must be produced using any of the processing methods 1 to 5 or processing method 7 (table 5).

#### *1.2.4.3 Gelatine for use in feed*

No data on ASFV survival in gelatine for use in feed has been identified in the literature review. The following raw material from pigs can be used for the production of gelatine intended for human consumption: bones and pig skins (Regulation (EC) No 853/2004, Section XIV of Annex III), and category 3

material can be used to produce gelatine suitable for animal consumption (Commission Regulation (EU) No 142/2011). In both cases, the process includes treatment with acid or alkali and extraction of gelatine by heating one or several times in succession (Table 7).

Table 7: Production process of gelatine (Regulation (EC) No 853/2004, Commission Regulation (EU) No 142/2011)

| Treatment of material | Extraction of gelatine | Purification | Further processing | Preservatives |
|---|---|---|---|---|
| treatment with acid or alkali, followed by one or more rinses; adjustment of pH | heating one or several times in succession | filtration and sterilisation | drying, pulverisation or lamination | sulphur dioxide, hydrogen peroxide |

#### *1.2.4.4 Collagen for use in feed*

No data on ASFV survival in collagen for use in feed have been identified in the literature review. Collagen for use in feed must be produced by a process ensuring that unprocessed Category 3 material is subjected to a treatment involving washing, pH adjustment using acid or alkali followed by one or more rinses, filtration and extrusion. After that treatment, collagen may undergo a drying process (Commission Regulation (EU) No 142/2011).

#### *1.2.4.5 Blood products for use in feed*

No data on ASFV survival in blood products for use in feed have been identified in the literature review. Blood products for use in feed must be submitted to any of the processing methods 1 to 5 or processing method 7 (Commission Regulation (EU) No 142/2011) (table 5). In a study funded by the European Association of Blood Products Producers (EAPA), Blazquez et al. (2018) inoculated liquid concentrated porcine plasma (28% solid) with ASFV to a final TCID50 concentration of 105.77 per mL of liquid concentrated plasma and found that spray-drying with an inlet temperature of 200°C and a 80°C outlet temperature inactivated 4.11 ± 0.20 log10 TCID50/mL of the inoculated ASFV in spray-dried porcine plasma.

#### *1.2.4.6 Dicalcium phosphate and tricalcium phosphate of animal origin for use in feed*

No data on ASFV survival in dicalcium phosphate or tricalcium phosphate for use in feed have been identified in the literature review. According to Commission Regulation (EU) No 142/2011, dicalcium phosphate must be prepared from Category 3 material that has been finely crushed and degreased with hot water and treated with dilute hydrochloric acid (at a minimum concentration of 4 % and a pH of less than 1,5) over a period of at least two days. The thus obtained phosphoric liquor must be treated with lime, resulting in a precipitate of dicalcium phosphate at pH 4 to 7, which has to be air-dried with an inlet temperature of 65 °C to 325 °C and an end temperature between 30 °C and 65 °C.

Tricalcium phosphate must be prepared from Category 3 material that has been finely crushed and degreased in counterflow with hot water (bone chips must be less than 14 mm). Subsequently, it has to be continuously cooked with steam at 145 °C during 30 minutes at 4 bars. The protein broth must be separated from the hydroxyapatite (tricalcium phosphate) by centrifugation and the tricalcium phosphate has to be granulated after drying in a fluidised bed with air at 200 °C.

Table 8 Production process of dicalcium phosphate and tricalcium phosphate (Commission Regulation (EU) No 142/2011)

| Product | Pre-treatment | Treatment 1 | Treatment 2 | Drying |
|---|---|---|---|---|
| Dicalcium phosphate | crushing<br>degreasing with hot water | treatment with dilute hydrochloric acid (minimum concentration 4 %, pH < 1,5) for at least 2 d | treatment with lime, resulting in a precipitate of dicalcium phosphate at pH 4 to 7 | air-drying (inlet temperature 65-325°C, end temperature 30-65°C |
| Tricalcium phosphate | crushing (bone chips must be < 14 mm)<br>degreasing in counterflow with hot water | continuous cooking with steam at 145 °C during 30 minutes at 4 bars | separation of protein broth from hydroxyapatite (tricalcium phosphate) by centrifugation | granulation after drying in a fluidised bed with air at 200°C |

## 2. ASFV survival in contaminated material

ASFV-infected domestic pigs and wild boar shed the virus through their excreta, such as faeces, urine, oral fluid (with or without blood). These excreta can contaminate other materials. It has been shown that ASFV survives in chilled (4°C) and cooled faeces (12°C) for at least 5 days, but not 7 days (Davies et al., 2017). In faeces stored at room temperature (21°C) the virus was shown to survive for at least 3 days, but less than 5 days (Davies et al., 2017), while an earlier study (Montgomery, 1921) found viable virus in faeces stored at room temperature (21°C) after 11 days. Faeces stored at 37°C were ASFV-negative 2 days after the start of the experiment (Davies et al., 2017).

The ASFV has been shown to survive in chilled (4°C) and cooled urine (12°C) for 5, but less than 7 days (Davies et al., 2017). In urine stored at room temperature (21°C) the virus was shown to survive for more than 5, but less than 7 days (Davies et al., 2017), while an earlier challenge study (Montgomery, 1921) found viable virus in urine stored at room temperature (21°C) for less than 2 days. Urine stored at 37°C was ASFV-negative 2 days after the start of the experiment (Davies et al., 2017).

A study conducted on slurry showed that slurry heated to 53°C in a reactor for 5.2-7.4 min did not contain active ASFV after this treatment (Turner et al., 1999) (table 9).

Table 9 Survival time of ASFV in excreta of infected domestic pigs or wild boar as reported in literature

| Matrix | Temperature range (°C) | Humidity range (%) | Maximum number of days ASFV was detected | First ASFV negative observation | Duration of the experiment in days | Half-life in days | LCI 95%[1] | UCI 95%[2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Faeces | Chilled (4°C) | | 5 | 7 | 98 | 0.65 | | | | Davies et al., 2017 |
| Faeces | Cooled (12°C) | | 5 | 7 | 98 | 0.5 | | | | Davies et al., 2017 |
| Faeces | Room temperature (21°C) | | 3 | 5 | 98 | 0.39 | | | | Davies et al., 2017 |
| Faeces | Room temperature (21–23°C) | | 11 | | 23 | | | | Challenge study, no virus isolation | Montgomery, 1921 |
| Faeces | Hot (37°C) | | 1 | 2 | 98 | 0.29 | | | | Davies et al., 2017 |
| Urine | Chilled (4°C) | | 5 | 7 | 126 | 2.19 | | | | Davies et al., 2017 |
| Urine | Cooled (12°C) | | 5 | 7 | 126 | 1.07 | | | | Davies et al., 2017 |
| Urine | Room temperature (15–25°C) | | <2 | | 2 | | | | Challenge study, no virus isolation | Montgomery, 1921 |
| Urine | Room temperature (21°C) | | 5 | 7 | 126 | 0.68 | | | | Davies et al., 2017 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Urine | Hot (37°C) | | 1 | 2 | 126 | 0.41 | | | | Davies et al., 2017 |
| Slurry | Heated (53°C) | | na | | Time in reactor 5.2-7.4 min | | | | Virus was inactivated below detectable levels after treatment in a reactor | Turner et al., 1999 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval; na=not applicable

## 2.1. Feed materials

Commission Regulation (EU) 2017/1017 provides a catalogue of feed materials. It also contains animal products. These must fulfil the requirements of the Regulation (EC) No 1069/2009 and Regulation (EU) No 142/2011 and may be subject to restrictions in use according to Regulation (EC) No 999/2001. This section lists only feed material that the AHAW Panel considers to be potentially contaminated with ASFV and that have not already been covered in previous sections. No studies were identified in the literature review that investigated the survival time of ASFV in feed material. The sections below list the production and processing parameters that could influence the potential survival of ASFV during these processes that were identified. The possibility of re-contamination after the production or processing process is beyond the scope of this section.

### 2.1.1. Cereal grains, their products and by-products

Dried distillers' grains with solubles that had been contaminated post-processing with ASFV and stored for 30 days at varying temperatures (mean 15°C) were found to be ASFV negative 30 days post contamination (Dee et al., 2018).

### 2.1.2. Oil seeds, oil fruits, their products and by-products

Soy oil cake, conventional soybean meal and organic soybean meal that had been contaminated post-processing with ASFV and stored for 30 days at varying temperatures (mean 12.3 or 15°C) were found to be ASFV positive 30 days post contamination (Dee et al., 2018; Stoian et al., 2019). The process of soybean meal includes several process steps, in which the raw material is heated (toasting by using dry heat to reduce or remove naturally occurring antinutritive factors). When leaving the toasting unit, the residual temperature is 105°C with 16-20% residual moisture (Witte, 1995) (table 10).

Table 10 Soybean meal production for animal feed (Witte, 1995)

| Product | Oil extraction | Solvent removal | Cooking | Drying & Cooling | Grinding & Sizing |
|---|---|---|---|---|---|
| Soybean meal | hexane-wet flakes leave the extractor at 53°C | desolventizing of extracted soy flakes with steam of 71-80°C | toasting of flakes (105°C at the exit, residual moisture 16-20%) | drying (45-75°C at exit, residual 12% moisture) cooling to 32°C (or ambient temperature +6°C) | size reduction by hammer or roller mills |

### 2.1.3. Legume seeds, their products and by-products

No data on ASFV survival in legume seeds, their products and by-products contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.1.4. Tubers, roots, their products and by-products

No data on ASFV survival in tubers, roots, their products and by-products contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.1.5. Other seeds and fruits, their products and by-products

No data on ASFV survival in other seeds and fruits, their products and by-products contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.1.6. Forages and roughage

No data on ASFV survival in forages and roughages contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review. Meals produced from certain forages, such as lucerne, clover or grass, are dried and milled. Hay stored in uncovered bales of different diameters and different moisture contents were shown to reach maximum temperatures of 77.2°C (Coblentz and Hoffman, 2009), bales covered in tarpaulin reached temperatures of 40.7-44.9°C, depending on location of storage and tarpaulin colour (Guerrero et al., 2010). In silage, during natural fermentation, the pH gradually drops and temperatures between 20-30°C are reached. The exact temperature and final pH in the ensiled crop largely depend on the type and moisture of the forage being ensiled. Maize silage terminates at or below pH 4, legumes silage generally reaches a terminal pH of about 4.5 (Seglar, 2013).

Table 11 Production parameters reported for hay and silage

| Matrix | Maximum temperature observed | Moisture concentrations (pre-storage) | pH | Reference |
|---|---|---|---|---|
| Hay bales uncovered | 77.2°C | 9.3-46.6% | | Coblentz and Hoffman, 2009 |
| Hay bales covered in tarpaulin | 40.7-44.9°C | | | Guerrero et al., 2010 |
| Silage | 20-30°C | | 4-4.5 | Seglar, 2013 |

### 2.1.7. Other plants, their products and by-products

This category contains cane molasses, cane vinasse, cane sugar and seaweed meal. No data on ASFV survival in these matrices contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

## 2.2. Compound feed

Compound feeding stuffs are organic or inorganic substances in mixtures, whether or not containing additives, for oral animal feeding in the form of complete feeding stuffs or complementary feeding stuffs (Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed). Feed contaminated with infectious material originating from infected domestic pigs or wild boar was found to be ASFV-positive for at least 5 days when stored at room temperature (22°C–25°C), at least for 30 days when stored at a temperature between 4°C and 6°C and at least 60 days when stored frozen (-16°C to -20°C) (Sindryakova et al., 2016). Dee et al. (2018) and Stoian et al. (2019) detected ASFV at day 30 post contamination in complete feed that had been contaminated post-processing with ASFV and stored for 30 days at varying temperatures (mean 12.3 or 15°C) (table 12).

## 2.3. Feed additives

Feed additives are substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to favourably affect the characteristics of the feed, the animal product, the colour of ornamental fish and birds, the environmental consequences of animal production and the animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, satisfy the nutritional needs of animals or have a coccidiostatic or histomonostatic effect.

Choline that had been contaminated post-processing with ASFV and stored for 30 days at varying temperatures (mean 12.3 or 15°C) was found to be ASFV-positive at day 30 post contamination (Dee et al. (2018); Stoian et al. (2019). The same authors did not detect ASFV 30 days post contamination of Lysine and Vitamin D that had been stored for 30 days at varying temperatures (mean 12.3 or 15°C) (table 12).

Table 12 Survival of ASFV in feed matrices contaminated with infectious material originating from infected domestic pigs or wild boar as reported in literature

| Matrix category | Matrix | Temperature range (°C) | Humidity range (%) | Maximum number of days infectious virus was detected | First ASFV negative observation | Duration of the experiment in days | Half-life in days | LCI 95%[1] | UCI 95%[2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cereal grains | Dried distillers' grains with solubles | Room (15°C (mean)) | 75 (mean) | 0 | | 30 | na | | | | Dee et al., 2018 |
| Oil seeds | Soy oil cake | Room (15°C (mean)) | 75 (mean) | 30 | | 30 | 5.0 | | | | Dee et al., 2018 |
| Oil seeds | Soy oil cake | Room (12.3°C (mean)) | 74.1 (mean) | 30 | | 30 | 12.4 | 10.4 | 14.3 | | Stoian et al., 2019 |
| Oil seeds | Soybean meal conventional | Room (15°C (mean)) | 75 (mean) | 30 | | 30 | 4.6 | | | | Dee et al., 2018 |
| Oil seeds | Soybean meal conventional | Room (12.3°C (mean)) | 74.1 (mean) | 30 | | 30 | 9.6 | 8.7 | 10.4 | | Stoian et al., 2019 |
| Oil seeds | Soybean meal organic | Room (15°C (mean)) | 75 (mean) | 30 | | 30 | 4.7 | | | | Dee et al., 2018 |
| Oil seeds | Soybean meal organic | Room (12.3°C (mean)) | 74.1 (mean) | 30 | | 30 | 12.9 | 11.5 | 14.3 | | Stoian et al., 2019 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound feed | Complete feed | Room (15°C (mean)) | 75 (mean) | 30 | | 30 | 4.3 | | | | Dee et al., 2018 |
| Compound feed | Complete feed | Room (12.3°C (mean)) | 74.1 (mean) | 30 | | 30 | 14.2 | 12.4 | 15.9 | | Stoian et al., 2019 |
| Compound feed | Feed | Frozen (-16 °C – -20°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| Compound feed | Feed | Chilled (4°C – 6 C) | | 30 | | 60 | | | | | Sindryakova et al., 2016 |
| Compound feed | Feed | Room (22°C –25 °C) | | 5 | | 60 | | | | | Sindryakova et al., 2016 |
| Feed additives | Choline | Room (15°C (mean)) | 75 (mean) | 30 | | 30 | 5.1 | | | | Dee et al., 2018 |
| Feed additives | Choline | Room (12.3°C (mean)) | 74.1 (mean) | 30 | | 30 | 11.9 | 10.9 | 12.9 | | Stoian et al., 2019 |
| Feed additives | Lysine | Room (15°C (mean)) | 75 (mean) | 0 | | 30 | na | | | | Dee et al., 2018 |
| Feed additives | Vitamine D | Room (15°C (mean)) | 75 (mean) | 0 | | 30 | na | | | | Dee et al., 2018 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval; na=not applicable

## 2.4. Tote bags

Tote bags are used for the transportation of feed grains. They are often re-used. Given the ability of ASFV to survive under a range of environmental conditions, the potential role of contaminated tote bags for ASFV spread should be assessed. No data on ASFV survival in tote bags have been found in the literature review.

## 2.5. Vehicles

Given the ability of ASFV to survive under a range of environmental conditions, the potential role of contaminated vehicles for spread of ASFV should be assessed.

### 2.5.1. Vehicles for live pig transport

No data on ASFV survival in vehicles used for live pig transport contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.5.2. Vehicles visiting pig farms

No data on ASFV survival in vehicles visiting pig farms contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.5.3. Other vehicles

No data on ASFV survival in vehicles other than those used for transporting live domestic pigs and those visiting pig farms contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

## 2.6. Bedding

### 2.6.1. Saw dust, wood chips

No data on ASFV survival in saw dust or wood chips contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review. Saw dust and wood chips are produced when wood logs are cut in sawmills. Stored in piles, self-heating may occur. The temperatures reached during self-heating depend on the amount of radiation, nutrient content of the wood or chips and their residual humidity. Temperatures in the piles may reach 60-80°C within 24 hours, with elevated temperature being maintained for weeks and ambient temperatures being reached after several months (Kofman, 2016).

### 2.6.2. Turf

No data on ASFV survival in turf (milled peat) contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar has been identified in the literature review. Milled peat collected from peat bogs during the dry season is stored in bales near collection fields or transported to storage sites. In Northern latitudes, the material is collected and stored outdoors in bales during summer months (commonly from May to September). After drying, the bales are often covered with plastic covers to protect them from rain and erosion, and to avoid self-ignition. Generally, a low pH (3.5-5) and temperatures of 40°C are reached in peat piles or bales (Mait Märtin, Elva EPT Ltd., personal communication).

### 2.6.3. Straw

No data on ASFV survival in straw contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

### 2.6.4. Hulls or husks of rice or other cereals

No data on ASFV survival in hulls of husks of rice or other cereals contaminated with ASFV or infectious material originating from infected domestic pigs or wild boar have been identified in the literature review.

## 2.7. Drinking water

Kovalenko et al. (1965) found that ASF virus in lake water that had been experimentally contaminated with blood from an infected domestic pig (dilution 1:100) and subsequently kept in a glass flask and buried at a depth of 12 cm survived for 50 days in summer and 176 days in winter.

Sindryakova et al. (2016) showed that water - that had been stored frozen (-16°C to -20°C), chilled (4°C–6°C) or at room temperature (22°C–25°C) - still contained viable ASFV at the end of the experiment (60 days) (table 13).

Table 13 Survival of ASFV in water contaminated with infectious material originating from infected domestic pigs or wild boar as reported in literature

| Matrix | Temperature range (°C) | Humidity range (%) | Maximum number of days infectious virus was detected | First ASFV negative observation | Duration of the experiment in days | Half-life in days | LCI 95%[1] | UCI 95%[2] | Comment | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Water | Summer | | 50 | | | | | | | Kovalenko et al. (1965) |
| Water | Winter | | 176 | | | | | | | Kovalenko et al. (1965) |
| Water | Frozen (-16°C – -20°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| Water | Chilled (4°C – 6°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |
| Water | Room (22°C – 25°C) | | ≥60 | | 60 | | | | | Sindryakova et al., 2016 |

[1]LCI 95% Half-life in days for the lower limit of the confidence interval; [2]UCI 95% Half-life in days for the upper limit of the confidence interval

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# Annex: Literature Review Protocol

**Objectives**

The overall aim of this review was to collect information from survival experiments published in primary research publications about African swine fever virus survival in different matrices.

**Review questions and eligibility criteria**

Outputs from agent survival studies

Collect data relating to the survival time of ASFV. Information should concern the persistence of the pathogen in different matrices.

***Review questions for agent survival studies***

What is the minimum and maximum number of days post inoculation that the pathogen (=viable ASFV) can be detected in different relevant matrices?

***Study eligibility criteria for agent survival studies***

| Element | Criteria | Level of screening |
|---|---|---|
| Publication type | • Primary research publications | Title and abstract |
| Language | • English | Title and abstract<br>Full-text |
| Study type | • Pathogen survival experiments | Title and abstract |
| Study characteristics | The study should provide details on<br>• the strain/isolate of the ASFV used<br>• the dose /quantity of virus used to infect/spike<br>• the temperature at which the matrix is stored during the experiment | Title and abstract<br>Full-text |
| Exposure | • Matrices from animals experimentally infected with ASFV OR<br>• Matrices experimentally contaminated (spiked) with ASFV | Title and abstract<br>Full-text |
| Outcome of interest | • The article is excluded if there is no description of the outcome of interest, i.e. ASFV survival time | Title and abstract<br>Full-text |
| Publishing date | Papers that have been published before 2019 and have been already included in the previous literature review will be excluded from data extraction. | Title and abstract |

**Methods for searching the results**

***Information sources***

The following databases were searched using the Web of Science (WoS) platform:

Web of Science Core Collection

- Science Citation Index Expanded
- Social Sciences Citation Index
- Conference Proceedings Citation Index- Science
- Conference Proceedings Citation Index- Social Science & Humanities
- Book Citation Index– Science
- Book Citation Index– Social Sciences & Humanities
- Emerging Sources Citation Index
- Current Chemical Reactions
- Index Chemicus

BIOSIS Citation Index

CABI : CAB Abstracts

Current Contents Connect

Data Citation Index

FSTA - the food science resource

KCI-Korean Journal Database (1980-present)

MEDLINE

Russian Science Citation Index

SciELO Citation Index

Zoological Record

***Restrictions***

Only primary research studies (i.e. no review papers) published in English were considered for potential inclusion in the reviews. The limitations concerning the year of publication listed above were applied.

Concerning the publications status, all literature indexed in the databases were included in the search, irrespective of whether they were e-pubs or corrected proofs.

**Reference management**

References were managed using the commercial reference management software package EndNote X9®. The articles were extracted and saved as an RSIS file for input into Distiller.

**Search strategy**

Ad hoc combinations of search terms were applied. The use of Boolean operators (AND, OR, NOT), truncation ($) and wildcard (*) symbols assured that search terms account for synonyms, abbreviations and spelling variants, enhancing thus the sensitivity of the search strategy.

Alternative names for ASFV were searched.

The objectives were searched using WOS (All databases), selecting from 2019 onwards and English only articles.

Publications were retrieved combining terms to represent the pathogen AND terms to describe survival experiment as follows:

| Set | Query |
|---|---|
| # 4 | #2 AND #1<br>**Refined by: PUBLICATION YEARS:** ( 2019 ) |
| #3 | #2 AND #1 |
| # 2 | **TOPIC:** (Surviv* OR Persist* OR stability OR inactivat* OR disinfect*) |
| # 1 | **TOPIC:** ("African swine fever" OR "Warthog disease" OR "Warthog fever") |

Excluding publication date: papers that were published until and including 31 Dec 2018 were excluded.

**Methods for study selection**

***Selection procedure***

The level 1 selection process involved the screening of title and abstract to identify potentially relevant studies by one reviewer using a screening check list developed according to the eligibility criteria defined in section 2.1.2. If the information contained in the title or abstract was not relevant for the research objectives, the article was not selected for full text assessment. The first level of screening was performed using Distiller®. Publications judged to be relevant were automatically selected for further screening, while publications rejected were excluded. References without abstract were carried over to level 2 screening, unless the title was explicit enough to clearly understand lack of compliance to one or more eligibility criteria.

For experimental infection studies, the level 1 screening was followed by a refinement process, by adding an extra question about the pathogen strain and whether or not the host was immunised or treated. Only a single exposure with an 'outbreak' strain or a 'wild type' strain of the pathogen in a not-immunised or not-vaccinated or not-treated host was accepted.

Level 2 screening involved the screening of full text articles identified in level 1, one reviewer per study, based on reading the full text.

***Retrieval of full texts***

Attempts were made to obtain electronic versions of the full papers for all references that fulfil the eligibility and relevance criteria (i.e. those passing Level 1 screening). This work was partly conducted during the literature search. Further retrieval of full papers was done between level 1 and level 2 screenings.

***Documenting the selection***

The study selection process was fully documented in Distiller, allowing tracking and reporting of:

- Number of records identified through each electronic database or other source
- Total number of unique records (title/abstracts) identified through electronic search
- Number of records excluded after level 1 screening
- Records (full text) potentially eligible
- Number of records excluded after level 2 screening (by reason for exclusion)
- Final number of studies included in the review

**Methods for data collection**

***Collected information***

Data collection for agent survival studies

| Field name | Data type | Description | Required | Lookup |
|---|---|---|---|---|
| studyID | integer | unique ID to link all observations from the same study or experiment | **YES** | |
| studyGroupID | integer | Unique ID for the animal group, or sample group, within this study, being reported | | |
| refID | integer | unique ID linking to the source of the information in the database of reference management system | **YES** | |
| agent | string | Code agent of ASF | **YES** | PARAM |
| studyTargetSpecies | string | Susceptible species used in the study | **YES** | MTX |

| | | | | |
|---|---|---|---|---|
| sampUnitSize | integer | number of samples tested in the study | | |
| sampledMatrix | string | Tissue sampled for testing | | MTX |
| Temperature | integer | Degrees celsius | | |
| humidity | number | Humidity conditions (%) | | |
| anMethCode | string | Laboratory test used for analysis for virus, antibodies or antigens associated with ASF | | ANALYMD |
| anMatText | radio | Target of laboratory test | | Nucleic acid<br>Virus |
| maxDetect | number | Maximum number of days post inoculation to observe pathogen, antibody or nucleic acid | YES | |

***Tools for data collection***

Data collection was carried out according to the data models defined above. Appropriate data collection forms, for each of the objectives, were set up to ensure that data validity checks were performed during data collection. This enforced compliance of the parameters to the data type described above – for instance enforcing that some parameters were entered as numerical or setting minimum and maximum ranges.

Forms for data collection were set up using Distiller®, which enabled setting user-friendly pick lists for data entry, and the resulting collected being a standardized set of codes based on the data catalogue. The data collected was then exported into a Microsoft Excel® spreadsheet.

***Procedure for data collection***

One reviewer per study individually extracted data from studies that passed screening for relevance. Authors of primary studies were not be contacted to provide missing or additional data.